

Appl. No. 10/500,878
Response dated August 16, 2007
Reply to Office Action of July 6, 2007

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-4 (cancelled)

Claim 5 (currently amended): A method to determine quantitatively the dissociation rate constants (k_{off}) for a transient complex of a ligand with the target molecule comprising the steps of:

- a) identifying a ligand site obeying a two-state binding behavior in a transient complex of a ligand with a target molecule by
 - i) preparing a ligand with at least one atomic nucleus detectable by NMR;
 - ii) collecting NMR relaxation dispersion profiles for said nucleus at two or more magnetic fields;
 - iii) determining apparent transverse relaxation rates from said dispersion profiles of step ii);
 - iv) assigning resonance peaks to said nucleus of the ligand with one- and/or multi-dimensional NMR;
 - v) contacting the ligand of step i) with at least one concentration of a target molecule;
 - vi) for each contacting of said ligand with at least one concentration of said target molecule as defined in step v) collecting NMR relaxation dispersion profiles for said ligand contacted with said at least one concentration of said target molecule for every concentration of said target molecule at two or more magnetic fields;
 - vii) fitting said dispersion profiles obtained in step vi) by including the relaxation rates of step iii) and using a two-state exchange model independently for every nucleus, and independently or simultaneously for every concentration of the target molecule; and
 - viii) determining a ligand site obeying a two-state binding behavior based on feasibility of extracted R_{2b} and p_b parameters obtained by the fitting of step vii), wherein R_{2b} represents a nominal transverse relaxation rate of said ligand bound to the target molecule and p_b represents a nominal fraction of the bound ligand; and

Appl. No. 10/500,878
Response dated August 15, 2007
Reply to Office Action of July 6, 2007

b) extracting and recording k_{off} values for the ligand sites obeying two-site exchange mechanism, said k_{off} values being a measure of the affinity of a transient complex of the ligand with the target molecule.

Claim 6 (previously presented): A method according to claim 5, wherein the ligand is a polypeptide.

Claim 7 (previously presented): A method according to claim 5, wherein the ligand is a ^{15}N -enriched polypeptide.

Claim 8 (previously presented): A method according to 5, wherein the ligand is a mixture of polypeptides and/or molecules.

Claim 9 (previously presented): A method according to claim 5, wherein the target molecule is a protein or a protein assembly.

Claims 10-14 (cancelled)

Claim 15 (currently amended): The method of claim 5, wherein the NMR relaxation dispersion profiles are collected by a CPMG Carr-Purcell-Meiboom-Gill (CPMG) method.

Claim 16 (cancelled)

Claim 17 (currently amended): The method of claim 5, wherein the ligand of step i) has at least two detectable atomic nuclei.

Claim 18 (previously presented): The method of claim 5, wherein the ligand in step v) is contacted with at least two concentrations of a target molecule.

Claim 19 (currently amended): The method of claim 5, wherein the ligand is contacted with three concentrations of a target molecules.